

The Sex-Determining Mechanism in some North American *Cicindelidae (Coleoptera)*¹

by

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With 16 photomicrographs and 1 table.

INTRODUCTION

In two of her papers on the cytology of various Coleoptera, STEVENS described the chromosome complements in two species of *Cicindela*. In males of one, *C. pimeriana*, she found 20 autosomes and what appeared to be an XY sex-determining pair (1906); in males of the other, *C. tranquebarica* (= "*C. vulgaris*"), there were two fewer autosomes but similar sex chromosomes (1909). Females were not examined. The type of association between the sex chromosomes proved to be unique among beetles, for the Y lay in the crotch of the larger, bipartite X-chromosome, that is, in an apparent centric position. STEVENS reported that disjunction was prereductional, as in almost all beetles.

In the course of studying five other North American species, *C. purpurea*, *C. ancocisconensis*, *C. repanda*, *C. sexguttata*, and *C. punctulata*, GOLDSMITH (1919) re-examined *C. tranquebarica*. Counting two more chromosomes in the female than in the male of *C. tranquebarica*, *C. punctulata*, and *C. ancocisconensis*, GOLDSMITH was led to conclude that the two associated and unequal-

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sized sex chromosomes of the male went to the same pole at first anaphase and divided equationally at the second. He thus interpreted the sex-determining mechanism as being Xx (δ): $XXxx$ (φ) —a situation reported otherwise only in *Doryphora signaticollis* (WIEMAN, 1910)¹ but well established, a few years before, as occurring in spiders (PAINTER, 1914). As has previously been stated (SMITH, 1950), GOLDSMITH's paper is far from convincing, and, on the basis of a somewhat cursory examination of preparations from three North American species, SMITH (1953) felt inclined to favour STEVENS' interpretation pending a more detailed study of further material. This has now been completed.

In the meantime, GUÉNIN (1952), likewise unimpressed by the evidence compiled by GOLDSMITH, has published the results of his investigation of four European species, *C. campestris* L., *C. hybrida* L., *C. silvicola* Latr., and *C. lunulata* F. His convincing illustrations, although validating GOLDSMITH's claim that the female possesses two chromosomes more than the male, establish the sex-determining mechanism as $X_1X_2X_3Y$ (δ): $X_1X_1X_2X_2X_3X_3$ (φ). The present account will show, however, at least for *C. repanda*, *C. sexguttata*, and *C. tranquebarica*, that GOLDSMITH was actually in error not only in so far as the sex complex is concerned but also in the chromosome counts he gave, for the diploid numbers of males and females differ by only one. The sex-determining mechanism in each must therefore differ also from that found by GUÉNIN. It is X_1X_2Y (δ): $X_1X_1X_2X_2$ (φ).

MATERIAL AND METHODS

Adults of four species, *C. repanda* Dej., *C. tranquebarica* Hbst., *C. sexguttata sexguttata* Fab., and *C. scutellaris lecontei* Hald., were collected at Laniel, on the Quebec-Ontario border; near Ottawa, Ontario; or from the sand shores of Lake Michigan, near St. Ignace, Michigan. Gonads were both dissected and fixed in Smith's modification of Kahle's fluid or in three parts absolute ethyl alcohol: one part glacial acetic acid, small portions of testes or the tips of

¹ GUÉNIN and SCHERLER (1951) have presented well-documented evidence that WIEMAN's interpretation was almost certainly incorrect: it most likely is an XO (δ): XX (φ) species.

ovarioles then being squashed in 45 per cent acetic acid. The former fixative proved better for gonial divisions, whereas the latter, because it facilitates the spreading of the chromosomes, was preferred for spermatocyte stages.

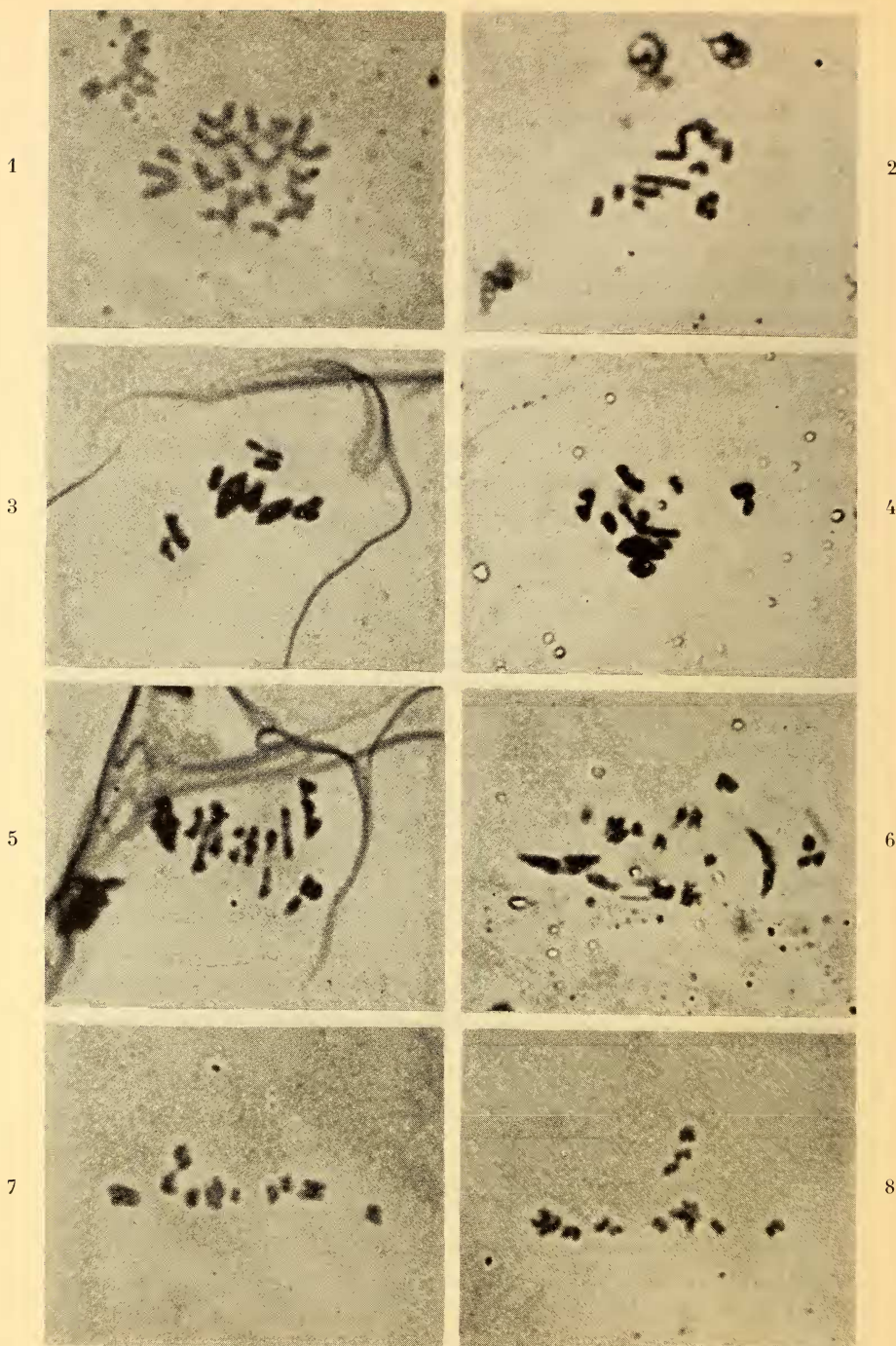
In studying males, a considerable saving in time and labour was achieved by uncoiling the individual testis, dissecting the long, tube-like gonad into a number of segments, and spacing the pieces systematically on slides before applying the cover slips. In this way the arduous task of searching for specific stages is considerably eased.

Staining was carried out using Feulgen's leuco-basic fuchsin and light green. Photomicrographs were taken on contrast process panchromatic film and printed on Kodabromide paper: their magnification herein is uniformly ca. $\times 1850$.

OBSERVATIONS

Both males and females were successfully examined in *C. repanda*, *C. tranquebarica*, and *C. scutellaris*, but in *C. sexguttata* males alone were available. The four species are numerically identical, with 21 chromosomes in males and 22 in those females studied. The chromosomes in spermatogonial metaphases cover a wide range in size and shape (ph. 1, 9, and 10) but the shape proved more difficult to ascertain, first, because the positions of the centromeres are open to certain delimitation only in the largest members of the complement and, second, because of considerable variability between cells of one and the same individual (cf. ph. 9 and 10 of *C. repanda*). As a consequence, no serious attempt has been made at "homologizing" pairs. However, each species obviously possesses four relatively large, more or less V-shaped chromosomes, a number of medium-sized ones, some of which are metacentric and others almost acrocentric, and several very short chromosomes, the shapes of which could not always be determined but nevertheless included obvious metacentrics (v. ph. 1, 9, and 10).

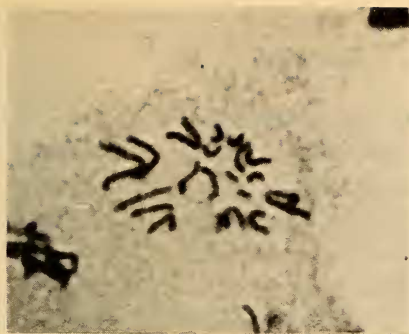
In under-contracted metaphases, a unique type of chromosome could often be identified in which a shorter, terminal portion is constricted off from the major part by a fine, tenuous thread that, in some instances, equaled in length the longer arm (ph. 10). It



PH. 1-8. — Spermatogenesis in *Cicindela tranquebarica*;
the sex complex, where identifiable, is to the right.

Ph. 1: Spermatogonial metaphase, $2n = 21$. — Ph. 2: Late pachytene, $9_{II} + XXY$. — Ph. 3-5: First metaphases; the nine bivalents have 12, 11 and 10 chiasmata respectively, and the three sex chromosomes show progressive stages of dissociation. — Ph. 6: First anaphase with the autosomes somewhat displaced by pressure and the XXY chromosomes just separated. — Ph. 7 and 8: Second metaphases with 10 and 11 chromosomes respectively.

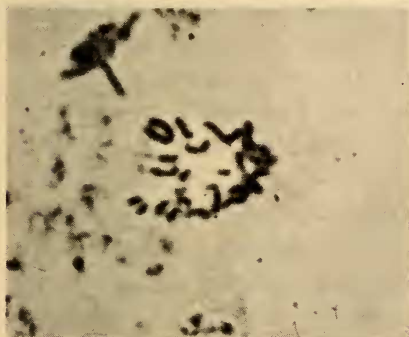
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10



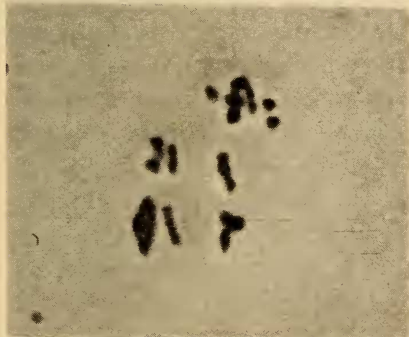
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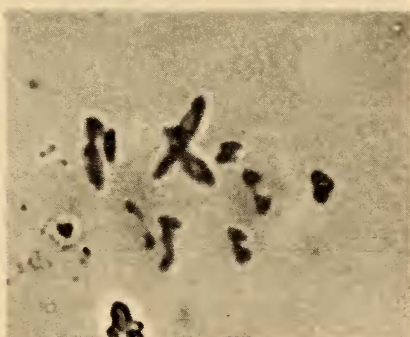
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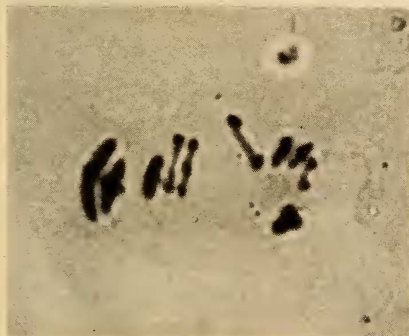
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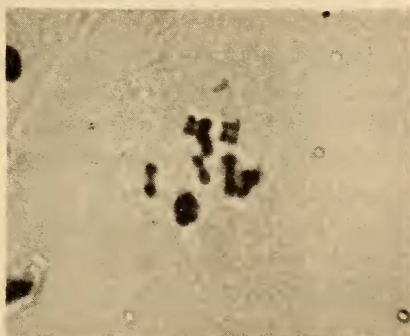
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15



16



PH. 9-16. — Meiosis in *C. repanda* (ph. 9-13), *C. sexguttata sexguttata* (ph. 14 and 15), and *C. scutellaris lecontei* (ph. 16); the sex complex, where identifiable, is to the right.

Ph. 9-10: Spermatogonial metaphases, $2n = 21$; note the satellited Y-chromosome in the centre of Ph. 10. — Ph. 11: Oogonial metaphase, $2n = 22$. — Ph. 12: Pachytene showing the large, strongly positively heteropycnotic, globular sex-complex. — Ph. 13-16: Primary spermatocyte metaphases, $9n + XXY$.

is presumably the nucleolar-organizing chromosome and, since it is unpaired and does not occur in the female, it might reasonably be taken to be the Y chromosome. Such a satellited chromosome was not discernible in *C. scutellatis*, but this might be attributed to the few metaphases seen being fully contracted.

In general, spermatogonial divisions occur with all later stages up to and including spermatids.

Oogonial metaphase complements (ph. 11) comprise 22 chromosomes similarly diverse in size and shape except for the absence of the satellited one. Like those of the spermatogonia, they are undistinguished by heteropycnosis, either positive or negative. Oocyte stages fail to stain with leuco-basic fuchsin and were thus not open to study.

Spermatocytes at pachytene are notable for their great frequency, a situation that establishes the long duration of this stage. They are most remarkable, however, even among Coleoptera, for the conspicuousness of the sex-determining complex. This is due to the intensity with which it stains and its extreme condensation (ph. 12). Even under pressures sufficient to expel it from among the autosomal bivalents, the complex remains essentially spherical and undifferentiated into its component chromosomes. Nine typical bivalents are formed, the number being readily established in later, well-flattened nuclei (cf. ph. 12 with GUÉNIN, 1952, fig. 12).

In contrast to the protraction of pachytene, transition to metaphase is an almost explosive process. Post-pachytene stages depicting the gradual opening-out of bivalents into the rings and crosses characteristic of typical diplotene and uncongressed yet condensed bivalents expected of diakinesis are accordingly not found even after extensive scrutiny.

First metaphases are usually abundant. There is almost always at least one ring-shaped bivalent with two unterminalized chiasmata (ph. 5): it is clearly formed by the largest pair of spermatogonial chromosomes. The next largest forms either a ring (ph. 3 and 15) or a rod (ph. 5) usually with unterminalized chiasmata. The remaining chromosomes mostly form rod bivalents, especially the smaller ones, and range down to relatively minute, compact, dumbbell-shaped pairs (ph. 5, 13, and 16). Thus, in general, the sizes and shapes of the bivalents at metaphase agree well with expectation based on the spermatogonial chromosomes.

From early to full metaphase, the sex complex is usually a highly condensed tripartite configuration (ph. 3 and 14-16), most readily interpreted, as by STEVENS (1906 and 1909) and by SMITH (1953), as consisting of a smaller, round element inserted between the highly contracted arms of a larger one. That this is, in fact, incorrect is shown by the occurrence of anaphases with unequal numbers of chromosomes in progression to opposite poles. Unfortunately, such transitional stages are but rarely seen, presumably because anaphase is of short duration. In the clearest examples encountered, however, there appears to be no doubt that, in agreement with spermatogonial counts, there are 11 chromosomes passing to one pole and 10 to the other. These counts are further substantiated by the occurrence of the two comparable types of second metaphases (ph. 7 and 8).

Late metaphases (ph. 4, 13, and 5) or early anaphases (ph. 6)—the chromosomes of the largest bivalents maintain their association longer than those of the others—reveal quite clearly that the sex complex is indeed composed of three roughly spherical bodies arranged as at the three corners of a triangle. Association is usually closer between the two oriented towards one pole, and one of the two is often alone joined by a Feulgen-positive thread to the third component (see especially ph. 5). In size the three differ but little; in arrangement they ensure a 2:1 disjunction (ph. 6). This disjunction is well co-ordinated with that of the largest chromosomes (cf. GUÉNIN, 1952, figs. 6 and 7 with ph. 6 herein; *contra* GOLDSMITH, 1919).

DISCUSSION

It will be evident that the present study does much to clarify the considerable confusion existing in accounts of the chromosome numbers and sex-determining mechanisms found in the genus *Cicindela*. Results published to date are summarized and compared with our current findings in Table I. By and large, the differences in opinion expressed by STEVENS and GOLDSMITH are best attributed to the pioneer nature of their work, the considerable difficulties inherent in the material, the inferiority of their techniques, and to some extent personal bias (see also SMITH, 1950 and 1953).

GUÉNIN has pointed out that GOLDSMITH's results are in perfect agreement with his own if the sex-determining mechanism in the North American species, instead of being $Xx:XXxx$, is of the European type. However, it is manifestly clear from the present observations that GOLDSMITH was in error on both counts, sex-determining mechanism and chromosome number, at least for *C. tranquebarica*, *C. repanda*, and *C. sexguttata*. Moreover, in view of GOLDSMITH's statement "One of the most perfect fixations (*C. sexguttata*) was one of seventeen specimens collected, dissected, and treated under the same conditions . . . The other sixteen were absolutely worthless" it is well-nigh inconceivable that his determinations for the other species are free from error.

GUÉNIN (1952), in comparing the spermatogonial chromosomes of his *C. campestris* and *C. hybrida* with those of GOLDSMITH's *C. sexguttata*, states: "les caryogrammes bien qu'établis d'après des images qui ont été obtenues par des techniques histologiques différentes, se ressemblent beaucoup et ne révèlent microscopiquement aucune modification importante dans la morphologie des éléments". This, we feel, is an overstatement since, apart from the two largest pairs, between only one of which is there good agreement, the comparison amounts to little more than an arrangement of graded series of chromosomes in decreasing order of size. In any case, the fact that GOLDSMITH's count for his species is actually wrong obviously renders an attempt to homologize karyotypes of dubious value. Nevertheless, there are several points of agreement between GUÉNIN's fig. 14 and the spermatogonia depicted herein. It will be noted in particular that the satellited chromosome observed here to be restricted to the male sex has been described by GUÉNIN as the Y chromosome in all four of the European species he studied. Such a uniform morphology is truly remarkable in view of the profusely documented evidence of the dispensability of the Y chromosome in other Coleoptera (see, for example, the compilation of X0 and neo-XY species in the order, SMITH, 1953). That it has persisted externally unchanged over the ages during which the North American and European species have been isolated is even more surprising than that the two geographical entities should possess "chromosomes sexuels multiples" (GUÉNIN, 1952). The Y, then, clearly plays a fundamental role, both as a whole and through its parts, in ensuring an orderly disjunction of

TABLE I.
Summary of cytological determinations in Cicindela species.

Species	$\delta/\varnothing$ counts and sex-determining mechanisms according to various authorities				
	STEVENS	GOLDSMITH	GUÉNIN	SMITH	HEREIN
<i>purpurea</i>		$\left\{ \begin{array}{l} 22/24 \text{ Xx: XXxx} \end{array} \right.$			
<i>ancociconensis</i>					
<i>punctulata</i>					
<i>pimeriana</i>	20/— XY: XX				
<i>tranquebarica</i>	22/— XY: XX	22/24 Xx: XXxx			21/22 XXY: XXXX
<i>serguttata</i>		22/24 Xx: XXxx		22/— Xy: XX	21/— XXY: XXXX
<i>scutellaris</i>				ca. 20/— Xy: XX	21/22 XXY: XXXX
<i>repanda</i>		22/24 Xx: XXxx		20/— Xy: XX	21/22 XXY: XXXX
<i>campestris</i>			$\left\{ \begin{array}{l} 22/24 \text{ XXXY: XXXXXX} \end{array} \right.$		
<i>hybrida</i>					
<i>silvicola</i>					
<i>lunulata</i>					

the component elements in spermatogenesis. Its obligatory absence from female meiosis is presumably compensated for by particulate association between the different X chromosomes.

GUÉNIN has concluded that the pairing between X_1 , X_2 , X_3 , and Y is independent of chiasmata and is the result of "stickiness". We are not prepared to commit ourselves on this point. GUÉNIN is then led to favour an origin of the three X chromosomes through fragmentation rather than through the incorporation of autosomal elements. It is conceivable, however, that the complex includes original autosomal components that were incorporated by fusion of the Y with one of a pair of autosomes and that the two autosomal elements have retained their original pairing specificity. The sex-determining mechanism could then logically be formulated here as $\widehat{XAAY}:XAXA$. Against this view is (1) the retention of the basic number of autosomes, 18, by both the European and North American species and (2) the positive heteropycnosis of the whole complex, for in beetles generally it is usual for neo-Y chromosomes and the autosomal parts of neo-X chromosomes to remain euchromatic (SMITH, 1952*a* and *b* and unpub.). Quite obviously the species under investigation offer no unequivocal evidence one way or the other. They do, however, constitute the hypothetical stage intermediate between the XY of the Coleopteran "basic formula" (SMITH, 1950 et al.) and the $X_1X_2X_3Y$ characteristic of the Old World species. It is tempting to speculate whether the other North American genera in the Cicindelidae, *Omus* Esch., *Amblycheila* Say, and *Tetracha* Hope, might not throw light on this question: material of any or all of these is accordingly solicited.

SUMMARY

Contrary to earlier reports, four North American species of *Cicindela* have 21 chromosomes in the male, 22 in the female. Their sex-determining mechanism is X_1X_2Y (σ): $X_1X_1X_2X_2$ (ϕ), as opposed to $X_1X_2X_3Y$: $X_1X_1X_2X_2X_3X_3$ in the four European species examined to date. Despite its presumed genetical inertness and consequent dispensability, the Y chromosome of the North American and European species has persisted morphologically unchanged throughout the period of isolation that has separated them geo-

graphically. The North American species constitute an evolutionary link between the XY sex-determining mechanism basic to Coleoptera as a whole and that of the European Cicindelids.

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